Calcitonin Inhibition of Gastric Secretion in Rat

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Synopsis

The recent discovery of the stimulatory effect of gastrin or pancreozymin on the secretion of calcitonin in the pig has prompted us to study the gastrointestinal effect of calcitonin in rats.

The marked increase in the gastric acid and pepsin accumulation in rats in which the pylorus was ligated for 9 hr was significantly inhibited by the prior treatment with 2 MRC U of purified porcine calcitonin.

This inhibitory effect of porcine calcitonin on the gastric acid and pepsin accumulation was dose dependent. Furthermore, the development of acute gastric ulcer in these rats was completely prevented by the s.c. administration of 2 MRC U of porcine calcitonin.

It has been considered that calcitonin is mainly concerned with the regulation of bone metabolism in mammals (Hirsch and Munson, 1969). However, the recent discovery of the stimulatory effect of gastrin or pancreozymin on the secretion of calcitonin in the pig (Cooper et al. 1971, Care et al. 1971) and the inhibitory effect of calcitonin on the gastric secretion in man (Hesch et al. 1971) has raised the possibility that calcitonin acts on the gastrointestinal organs. In view of these facts, we have studied the effect of calcitonin on the gastric secretion and furthermore on the development of experimental gastric ulcer in rat.

Materials and Methods

Exp. 1
Forty male Wistar rats of 250 gm b.w. were fasted 48 hr and randomly divided into the following equal 5 groups. Group I: control rats, Group II: rats, in which the pylorus was ligated for 9 hr, Group III: rats, in which the pylorus was ligated for 9 hr and given s.c. injection of 1 MRC U of purified porcine calcitonin before and 6 hr after the ligation of the pylorus, Group IV: rats, in which the pylorus was ligated for 6 hr, Group V: rats, in which the pylorus was ligated for 9 hr and given s.c. injection of 1 MRC U of porcine calcitonin 6 hr after the ligation of the pylorus. All 40 rats in Exp. I were done on the same day. All the rats, except Group I and Group IV were sacrificied 9 hr after the ligation of the pylorus. Group I was sacrificied at 0 time and Group V at 6 hr after the ligation of the pylorus. Blood samples were taken from each animal when they were sacrificied.

Exp. 2
Twenty eight male Wistar rats of 250 gm. b.w. were fasted 48 hr and randomly divided into the equal 4 groups. Group I: control rats, in which the pylorus was ligated for 6 hr, Group II, III & IV: rats, in which the pylorus was ligated for 6 hr and given s.c. injection of 0.0625, 0.25 and 1.0 MRC U of porcine calcitonin respectively before the pyloric ligation. All the rats were sacrificied 6 hr after the ligation of the pylorus.

Exp. 3
Twenty one male Wistar rats of 250 gm b.w. were randomly divided into the equal 3 groups. Group I: control rats without any treatment, Group II: rats, in...
which the pylorus was ligated for 9 hr. Group III: rats, in which the parathyroid gland was removed by surgery 48 hr prior to the experiment and the pylorus was ligated for 9 hr. All the rats except in Group I were sacrificed 9 hr after the ligation of the pylorus.

Blood samples and gastric juice were obtained from each animal when they were sacrificed for the determination of serum Ca, gastric acidity. In Experiment I, stomachs were taken out from each animal and the formation of acute ulcer was examined histologically. Serum Ca was determined by the colorimetric method of Webster (1962).

Gastric acidity was measured by the titration with 0.1N NaOH and gastric pepsin activity by Anson’s method. The significance of the difference in each experiment was tested by using student t-test (one-tailed).

Results

Exp. I

1. Effect of porcine calcitonin on serum Ca in pylorus ligated rat

In Table 1 is shown that effect of porcine calcitonin on the serum Ca in pylorus ligated rats. Ligation of the pylorus for 9 hr did not cause any significant change in serum Ca. Injection of 1 MRC U of porcine calcitonin before and 6 hr after the ligation of the pylorus caused a marked fall in serum Ca in pylorus ligated rats (P < 0.001). Injection of 1 MRC U of porcine calcitonin 6 hr after the ligation of the pylorus also caused a significant fall in serum Ca 9 hr after the ligation of the pylorus (P < 0.001).

2. Effect of porcine calcitonin on the accumulation of gastric acid in pylorus ligated rat

In Figure 1 is shown the effect of porcine calcitonin on the accumulation of gastric juice and acid in pylorus ligated rats. In this and succeeding figures, the accumulation of gastric juice stands for the amount of gastric acid accumulated (concentration of gastric acid x volume) during the pyloric ligation. In normal rats, in which the pylorus was not ligated, there was practically no accumulation of either gastric juice or gastric acid. On the other hand, in control rats, in which the pylorus was ligated, the accumulation of gastric juice and acid was markedly increased during 9 hr pyloric ligation. However, these increases were significantly inhibited by 1 MRC U of porcine calcitonin injected before and 6 hr after the ligation of the pylorus (P < 0.001). Furthermore, calcitonin injected 6 hr after the ligation of the pylorus also inhibited the accumulation of both gastric juice and acid in these rats (P < 0.001).

3. Effect of porcine calcitonin on gastric pepsin accumulation in pylorus ligated rat

In control rats, ligation of the pylorus for 9 hr caused a marked increase in the gastric pepsin accumulation, as shown in Figure 2. In this and succeeding figures, the gastric pepsin accumulation stands for the amount of pepsin accumulation (pepsin activity x volume) during the pyloric ligation. Porcine calcitonin, injected before and 6 hr after the ligation of the pylorus significantly inhibited the increased pepsin accumulation caused by the ligation of the pylorus (P < 0.001) (Fig. 2).

4. Inhibitory effect of porcine calcitonin on the development of acute gastric ulcer in pylorus ligated rat

In Figure 3 is shown the effect of porcine calcitonin on the development of acute gastric
Fig. 1 Calcitonin inhibition of the accumulation of gastric juice and acid in pylorus ligated rat
The marked increase in the accumulation of gastric juice and acid in rats 9 hr after the ligation of the pylorus was significantly inhibited by the s.c. injection of either 2 MRC U of porcine calcitonin injected at 0 and 6 hr or 1 MRC U of calcitonin injected at 6 hr after the pyloric ligation (P < 0.001).
In this and Figure 2, each bar represents the mean of 8 rats and horizontal line represents the standard error.

Fig. 2 Calcitonin inhibition of gastric pepsin accumulation in pylorus ligated rat
The marked increase in the gastric pepsin accumulation in rats 9 hr after the ligation of the pylorus was significantly inhibited by the s.c. injection of 2 MRC U of porcine calcitonin (P < 0.001).
ulcer in pylorus ligated rat. All the rats developed acute gastric ulcer 9 hr after the ligation of the pylorus. However, injection of 1 MRC U each of porcine calcitonin before and 6 hr after pyloric ligation completely prevented the development of ulcer formation. Two out of 8 rats developed gastric ulcer 6 hr after the ligation of the pylorus. Subcutaneous injection of 1 MRC U of porcine calcitonin 6 hr after the ligation of the pylorus prevented the further development of such ulcer formation.

**Exp. II**

1. **Effect of graded doses of porcine calcitonin on serum Ca in pylorus ligated rat**

   As shown in Table 2, there was a dose related decrease in serum Ca 6 hr after s.c. injection of graded doses of porcine calcitonin.

2. **Effect of graded doses of porcine calcitonin on the accumulation of gastric acid in pylorus ligated rat**

   Ligation of the pylorus caused a marked

*Fig. 3* Inhibitory effect of porcine calcitonin on the development of acute peptic ulcer in pylorus ligated rat

The development of acute gastric ulcer in pylorus ligated rat was completely prevented by s.c. injection of 2 MRC U of porcine calcitonin. One MRC U of porcine calcitonin injected 6 hr after the ligation of the pylorus prevented the further development of such gastric ulcer.

*Fig. 4* Dose related decrease in the accumulation of gastric juice and acid in pylorus ligated rats following s.c. injection of graded dose (0.0625, 0.25, 1.0) of porcine calcitonin.

In this and Figure 5, each point represents the mean of 7 rats and vertical line represents the standard error.
Table 2. Changes in the serum Ca of pylorus ligated rats 6 hr after s.c. injection of graded doses of porcine calcitonin.

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of rats</th>
<th>Serum Ca, mg/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (6 hr ligation)</td>
<td>8</td>
<td>0.4 ± 9.2*</td>
</tr>
<tr>
<td>Porcine CT, 0.0625 U</td>
<td>8</td>
<td>9.3 ± 0.3</td>
</tr>
<tr>
<td>Porcine CT, 0.25 U</td>
<td>8</td>
<td>8.9 ± 0.2</td>
</tr>
<tr>
<td>Porcine CT, 1.0 U</td>
<td>8</td>
<td>8.5 ± 0.2**</td>
</tr>
</tbody>
</table>

* > **, P < 0.01

increase in the accumulation of gastric juice and acid during 6 hr ligation in control rats. However, this increase was inhibited in a dose related manner following the s.c. injection of graded doses of porcine calcitonin (Fig. 4).

3. Effect of graded doses of porcine calcitonin on the gastric pepsin accumulation in pylorus ligated rat

As shown in Figure 5, gastric pepsin accumulation was markedly increased in control rats in which the pylorus was ligated for 6 hr.

Subcutaneous injection of graded doses of porcine calcitonin significantly decreased the pepsin accumulation in a dose related manner (Fig. 5).

Exp. III
Effect of parathyroidectomy on serum Ca and gastric acid accumulation in pylorus ligated rat

In Figure 6 is shown the changes in the serum Ca and gastric acid accumulation in pylorus ligated rats after parathyroidectomy. The increased gastric acid accumulation in pylorus ligated rat was not inhibited by parathyroidectomy despite the marked fall in serum Ca in these rats.

Discussion

The present study clearly demonstrated that porcine calcitonin markedly inhibited the gastric acid accumulation and furthermore the development of acute gastric ulcer in pylorus ligated rat. Recently, Hotz et al. (1971) have shown that porcine calcitonin caused a fall in gastric secretion in rats with chronic gastric fistula. The inhibitory effect of calcitonin on gastric secretion has also been demonstrated in man. Hesch et al. (1971) have shown a marked inhibition of both basal and pentagastrin stimulated gastric secretion in 8 normal men following a single injection of salmon calcitonin. We have recently found that a continuous infusion of porcine calcitonin caused a marked fall in tetragastrin stimulated gastric acid secretion in 8 patients with peptic ulcer (Orimo et al., 1972). Furthermore it was also found that porcine calcitonin inhibited the histamine stimulated increase in gastric secretion in man (Orimo et al., 1972). Of particular interest is the mechanism of inhibitory effect of calcitonin on the gastric acid secretion. It has been shown that hypercalcemia stimulates and hypocalcemia inhibits the gastric acid secretion in man through the change in the secretion of gastrin (Barreras and Donaldson,
Effect of Parathyroidectomy on Serum Ca and Gastric Acid Secretion in Shay's Rat

Fig. 6 Failure of parathyroidectomy in preventing the increase of gastric acid accumulation in pylorus ligated rats despite the fall in serum Ca.
In this figure, each column represents the mean of 7 rats and horizontal line represents the standard error.

1967; Donegan and Spiro, 1960; Trudeau and McGuigan, 1969; Reeder et al., 1970). However, in dogs, hypercalcemia is reported to inhibit the gastric acid secretion. In rat, it has been shown that a marked inhibition of gastric acid secretion was caused by either hypercalcemia or hypocalcemia (Hotz et al., 1971; Kowalewski, 1968). According to our experiment, gastric acid output in parathyroidectomized rat is not significantly different from that in intact rat, which suggests that hypocalcemia did not necessarily cause the inhibition of gastric acid secretion in rat. Furthermore, the inhibitory effect of calcitonin on the gastric secretion in man is quite marked in the absence of hypocalcemia. As shown in Figure 4, lower dose of porcine calcitonin (0.0625 MUC U) caused a significant inhibition of gastric acid accumulation in pylorus ligated rats 6 hr after the s.c. injection when the hypocalcemic effect of calcitonin has already disappeared. These results may suggest that the inhibitory effect of calcitonin on the gastric acid secretion may be due to a direct effect of porcine calcitonin and may not be due to the hypocalcemia caused by this hormone, although it may be difficult to prove this definitely. Particularly interesting is the fact that acute gastric ulcer in rat in which the pylorus was ligated for 9 hr was completely prevented by the prior treatment with calcitonin. However, if the time after ligation of the pylorus is extended to 12 hr the inhibitory effect of calcitonin on the development of peptic ulcer is markedly decreased (preliminary observation). Since it has been suggested that acute gastric ulcer in pylorus ligated rat is produced by the increased secretion of gastric acid (Sun and Chen, 1963), it is possible that calcitonin inhibited the development of acute gastric ulcer by the inhibition of gastric acid secretion. These findings, together with our observation that calcitonin inhibits the gastric acid secretion in patients with peptic ulcer, suggest that calcitonin may be useful in preventing the development of
peptic ulcer.

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References